Preliminary investigations of glyphosate metabolism in resistant and susceptible plants have indicated that glyphosate is not rapidly metabolised by either biotype.

Application of glyphosate causes rapid accumulation of shikimate in treated plants and unregulated diversion of carbon may be the major reason for the herbicidal effect of glyphosate.[2] We therefore carried out an experiment to examine whether shikimate accumulates to any lesser extent in resistant plants. Plants were treated with 112.5 g ha^{-1} glyphosate and then, at intervals up to seven days, sets of 12 plants were pooled and analysed for shikimate.[5] Figure 1 shows that, following an initial similar rise, there was consistently lower accumulation of shikimate in treated resistant plants than in treated susceptible plants. Significantly, shikimate levels returned to pre-treatment concentrations after seven days in the resistant population, but not in the susceptible population. This would seem to be a very notable factor, underpinning the eventual survival of the resistant plants. Indeed the dynamics of the changes in shikimate concentration do seem to parallel the whole plant situation, where initial stunting is observed in the first few days, followed by survival and regrowth.

The above results presented a dilemma. There were no major differences in either the target pathway or in rates of uptake/translocation of glyphosate between the two biotypes. Nevertheless, on the basis of substrate accumulation, the degree of inhibition experienced by resistant plants was clearly reduced. Does less glyphosate reach the target site in resistant plants? The observation of significant cross-resistance (*c*4-fold, data not shown) to a structural analogue of glyphosate, 2-hydroxy-3-(1,2,4-triazol-1-yl)propyl phosphonate (TP) suggested that this may indeed be the case. TP is an experimental herbicide with a mode of action (inhibition of histidine biosynthesis) completely unrelated to that of glyphosate, but, like EPSP synthase, it also acts at a target site (imidazole glycerol phosphate dehydratase) located exclusively within the plastid.[6]

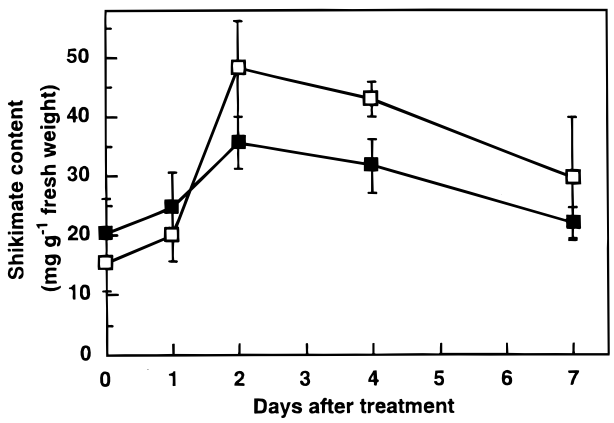

Figure 1. Shikimate accumulation in shoots of (□) susceptible and (■) glyphosate-resistant *Lolium rigidum* plants following the application of 112.5 g AE ha^{-1} glyphosate to two-leaf stage plants. Data are means (±S.E.) of six replications.

Significantly, there was no cross-resistance to more distantly related structures (eg phosphinothricin; see also Reference 1). It is thus tempting to speculate that at least one component of resistance (which may, of course, be multi-factorial) arises from reduced movement of glyphosate to its site of action in the plastid. Glyphosate and TP are structurally similar to phosphate esters shuttled between the plastid and cytoplasm. Movement of the herbicides may therefore be dependent upon the same molecular carriers as involved in the shuttles. Resistance to glyphosate based upon a mutation in such a carrier would be expected to be inherited as a recessive or partially dominant trait. Preliminary experiments indicate that this is indeed the case. We are currently looking at herbicide uptake into chloroplasts and examining the inheritance of resistance in more detail.

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Synthesis of analogues of the monic acids A and C as potential herbicides

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Abstract: The synthesis of some analogues of pseudomonic acids with simplified 'left-hand' side-chains is reported. Tested against isoleucyl tRNA, none was as active as methyl monate A and none was active as a herbicide in glasshouse tests.

Keywords: pseudomonic acid; monic acid; herbicide; herbicidal activity; isoleucyl tRNA synthetase

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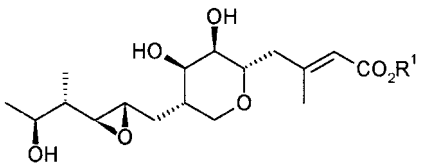
The pseudomonic acids A, B, C and D are a family of naturally occurring antibiotics produced by fermentation of a strain of *Pseudomonas fluorescens* (Trev) Mig.[1–4] Pseudomonic acid A (Fig 1; **1**), the major metabolite, has a broad spectrum of activity against Gram-positive bacteria and is marketed as 'Bactroban' by SmithKline Beecham for topical use in the treatment of skin infections. It acts through the inhibition of bacterial protein synthesis by binding to isoleucyl tRNA synthetase.[5] Pseudomonic acid C (**2**) has an olefinic bond in its 'left-hand' (ie position 5 of the tetrahydropyran ring) side-chain in place of the epoxide of pseudomonic acid A.

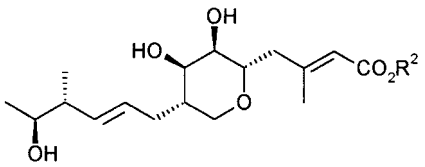
Hydrolysis of pseudomonic acids A and C gives the corresponding monic acids, **3** and **4**. It has previously been reported that various ester and amide derivatives of monic acid A are herbicidal,[6–8] and that this biological activity also stems from inhibition of isoleucyl tRNA synthetase. Methyl monate A is an example of a derivative of this kind. It controls the growth of a variety of broad-leaved weeds in the glasshouse when applied post-emergence at a rate of $100\,g\,ha^{-1}$, and has $IC_{50} < 1\,nM$ against isoleucyl tRNA synthetase isolated from spinach chloroplasts. 2-(Vinyloxy)ethyl monate A (**5**) is even more active as a herbicide, and was taken forward into extensive field trials. In the best of these, it gave good control of several important weeds at rates of $25–50\,g\,ha^{-1}$, but its performance varied depending on the growth stage and species of weed, as well as on environmental conditions.[8]



$R^1 = (CH_2)_8CO_2H$: Pseudomonic Acid A **1**

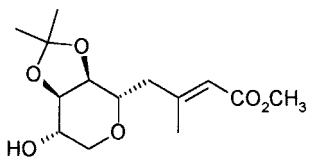
$R^1 = H$: Monic Acid A **3**

$R^1 = (CH_2)_2OCH{:}CH_2$ **5**



$R^2 = (CH_2)_8CO_2H$: Pseudomonic Acid C **2**

$R^2 = H$: Monic Acid C **4**



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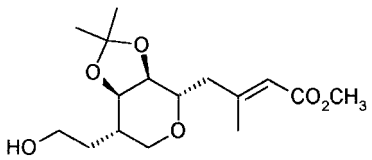

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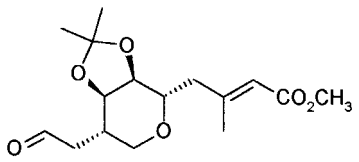

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Figure 1. Structures of compounds discussed.

In addition to the problems outlined above, any derivative of a monic acid would have to be manufactured by synthetic modification of a fermentation product, and this is likely to be an expensive procedure. We were therefore interested to know whether simplified, fully synthetic analogues of the monic acids could be designed and used as herbicides. This summary describes how we have synthesised analogues of methyl monates A and C with simplified 'left-hand' side-chains to see if herbicidal activity is retained. We chose the 'left-hand' side-chain because most previous studies, mainly by chemists at SmithKline Beecham, have focused on the 'right-hand' side-chain which is more straightforward to modify starting from a supply of the natural product, and where changes can be made without loss of antibacterial activity. Indeed, since the time when the structure of pseudomonic acid A was first described[1] there has been a steady stream of patent applications claiming analogues of the pseudomonic acids with modified 'right-hand' side-chains as antibacterial compounds.

We are aware of just two papers and three patent applications describing changes to the 'left-hand' side-chain of the monic and pseudomonic acids, all from chemists at SmithKline Beecham. In the first of the papers, various changes to the hydroxyl group (removal, epimerisation, replacement with a chlorine atom or an amino group) in monic and pseudomonic acids and esters A and C were described, all leading to a marked reduction in antimicrobial activity.[9] The second paper was published while we were performing the chemistry reported below, and described compounds in which more substantial alterations to the 'left-hand' side-chain had been made.[10] Again, all of these changes led to compounds with very weak activity against both isoleucyl tRNA synthetase and bacteria. The first patent application claims compounds in which the hydroxyl group has been oxidised to the ketone,[11] while two others, published very recently, claim analogues with an amidosulfate group in the 'left-hand' side-chain.[12,13]

We chose to use three key intermediates in our studies, the alcohols **6** and **7**, and the aldehyde **8**. The alcohol **6** was prepared in homochiral form from (L)-lyxose, building on chemistry first described by Keck and his co-workers.[14] The alcohol **7** was prepared in racemic form by adaptation of chemistry

described by Snider and his co-workers.[15] Finally, the racemic aldehyde **8** was prepared by oxidation of the alcohol **7** with pyridinium chlorochromate. A closely related aldehyde, with an ethyl rather than methyl ester in the 'right-hand' side-chain and prepared in homochiral form by degradation of the pseudomonic acid A, was also chosen as an intermediate by the chemists at SmithKline Beecham.[10]

We synthesised a variety of derivatives of each of the three key intermediates. Hydrolysis of the acetonide protecting group then gave analogues of the methyl monates for testing against isoleucyl tRNA synthetase and in the glasshouse. Representative examples are shown in Figs 2, 3 and 4. All of these analogues were found to be much less active than methyl monate A, and most gave no significant inhibition of isoleucyl tRNA synthetase, even at micromolar concentrations. None was active in the glasshouse. Of all the compounds made, the olefins **9**, **10** and **11** shown in Fig 4 were the most potent inhibitors of the enzyme, and it is perhaps significant that these are close analogues of methyl monate C.

Having prepared and tested the compounds described above, we were less optimistic about finding any simplified analogues with high biological activity. However, it has been argued that the left-

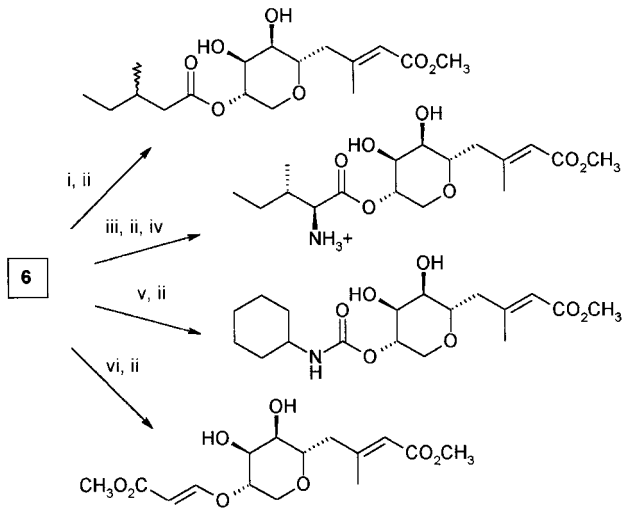

Figure 2. Synthesis of methyl monate analogues from intermediate alcohol **6**. i, 3-CH_3-pentanoic acid, DCC, DMAP; ii, aq. CH_3CO_2H; iii, *N*-*t*-BOC-L-isoleucine, DCC, DMAP; iv, CF_3CO_2H; v, cyclohexyl isocyanate, DBU; vi, methyl propiolate, *N*-CH_3-morpholine.

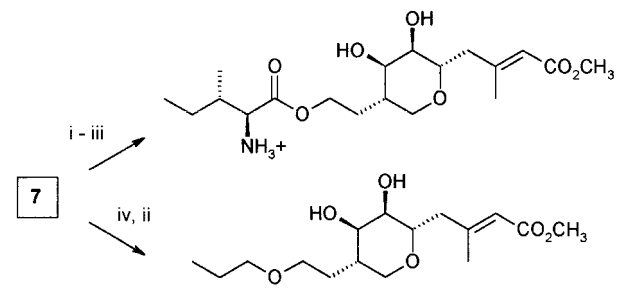

Figure 3. Synthesis of methyl monate analogues from intermediate alcohol **7**. i, *N*-*t*-BOC-L-isoleucine, DCC, DMAP; ii, aq. CH_3CO_2H; iii, CF_3CO_2H; iv, $CH_3(CH_2)_2OSO_2CF_3$, K_2CO_3.

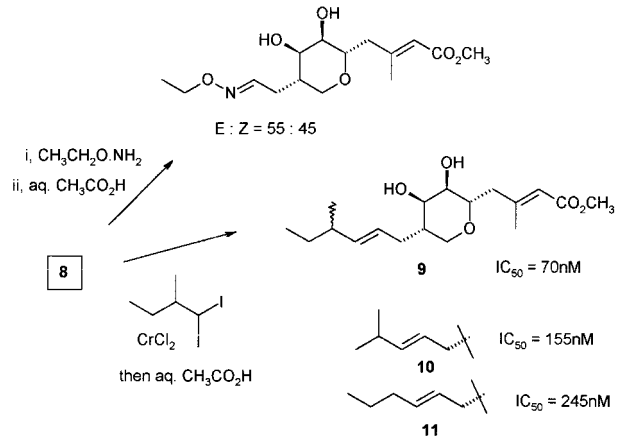

Figure 4. Synthesis of methyl monate analogues from intermediate aldehyde **8**.

hand end of the pseudomonic acids, which resembles isoleucine, is the key recognition unit at the binding site of isoleucyl tRNA synthetase.[5,16] If this is the case, we thought it was still possible that there might be a very limited number of analogues with high activity. Consistent with this idea is the observation that the olefin **9**, which most closely resembles isoleucine and has the complete side-chain of methyl monate C except for the hydroxyl group, is more active than the olefins **10** and **11** which are missing the terminal or branching methyl group, respectively (as well as the hydroxyl group), of methyl monate C.

We opted to prepare further analogues for testing, but as mixtures of 10 compounds per sample. In this way, we would make optimal use of time and our valuable intermediates. One example is shown in Fig 5. The mixture of 10 esters shown gave no significant inhibition of isoleucyl tRNA at a concentration of 10 μM (ie, 1 μM with respect to each component). When spiked with methyl monate A at the concentration of one of the 10 components, the mixture showed complete inhibition of the enzyme at 10 μM, suggesting that had an active compound been present, it would have been detected. Further libraries of esters of alcohols **6** and **7**, as well as ether derivatives of **7**, were also prepared, and again no activity was found.

In a relatively short time, we had shown that analogues of methyl monates A and C with a variety of modifications in the 'left-hand' side-chain are not good inhibitors of isoleucyl tRNA synthetase. Further related compounds with alternative func-

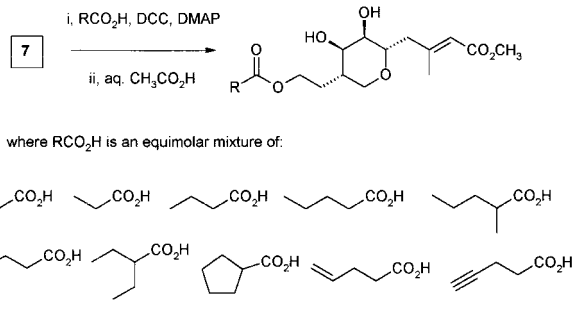

Figure 5. Synthesis of mixed methyl monate analogues.

tionality in the 'left-hand' side-chain can, of course, be written down. However, on the basis of the results above, we can conclude that there is much less scope for modification of the structure of methyl monates in the 'left-hand' side-chain than there is at the 'right-hand' side.

Finally it is worth noting that the mixtures approach described above was useful here because of a specific set of circumstances: (1) most, if not all, of the analogues in the series were very weak inhibitors or inactive; (2) there was an expectation, based on the potency of compounds with the natural 'left-hand' side-chain, that high activity could be achieved with a suitable synthetic compound; (3) an enzyme assay, with sensitivity over at least four orders of magnitude, was in place; and (4) the chemistry leading to the esters was high-yielding and sufficiently robust to work with mixtures of compounds (as, to an adequate extent, was the ether chemistry). Clearly, this approach is quite unsuitable for conventional series of analogues in which most or many have some activity.

ACKNOWLEDGEMENTS

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Insecticidal natural products: new rocaglamide derivatives from *Aglaia roxburghiana*

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Abstract: In the course of the screening for novel, naturally occurring pesticides from the plant family Meliaceae, an extract of the stem bark of *Aglaia roxburghiana* was found to exhibit significant insecticidal activity. In addition to rocaglamide, a known insecticide isolated from several species of the genus *Aglaia*, 15 new natural products were isolated from this plant. Isolation and structure elucidation of the natural products is described. The outstanding insecticidal activities of some of the compounds as well as a structure–activity relationship study are presented.

Keywords: *Aglaia roxburghiana*; meliaceae; plant metabolites; benzofurans; rocaglamide analogues; aglaroxins, insecticidal activity

1 INTRODUCTION

Plants provide an abundant source of secondary metabolites possessing biological activities in the crop protection area. In a few cases, either the natural products themselves (pyrethrin, rotenone, azadiracthin) or synthetically modified, but closely related, structures (pyrethroids) have reached the market place.

In recent years, the most detailed studies of the effects of a natural product on insect behaviour and physiology have been those carried out on the limonoid, azadirachtin, from the neem tree, *Azadirachta indica* Juss (Meliaceae).

Rocaglamide was isolated from *Aglaia elliptifolia* by King *et al*[1] by following the antileukemic activity against P388 lymphocytic leukemia in CDF1 mice. Simultaneously, Pachlatko and Kumar[2] discovered that rocaglamide, isolated from *Aglaia congylos*,

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